JHWCR

Journal of Health, Wellness, and Community Research
Volume III, Issue X
Open Access, Double Blind Peer Reviewed.
Web: https://jhwcr.com, ISSN: 3007-0570
https://doi.org/10.61919/pv9z4k05

Original Article

Association of Serum Calcium and Vitamin D Levels with Premenstrual Syndrome in Women from District Sialkot: A Case–Control Study

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Authors' Contributions: Concept and design: Concept: SA; Design: SA; Data Collection: SD, AS; Analysis: SD, AS, MA; Drafting: SA, MA, AI, SD
Cite this Article | Received: 2025-07-21 | Accepted 2025-08-04
No conflicts declared; ethics approved; consent obtained; data available on request; no funding received.

ABSTRACT

Background: Premenstrual syndrome (PMS) is a multifactorial condition with physical, emotional, and behavioural manifestations that significantly impair women's quality of life. Emerging evidence suggests that micronutrient deficiencies, particularly calcium and vitamin D, may play a central role in PMS pathophysiology. Objective: To investigate the association between serum calcium and vitamin D levels and PMS among reproductive-aged women in District Sialkot. Methods: A case–control study was conducted involving 40 women diagnosed with PMS and 40 age-matched controls without PMS. Participants underwent structured symptom assessments and venous blood sampling during the luteal phase. Serum calcium was analyzed using the Ortho-Cresolphthalein Complexone method, while vitamin D was quantified by chemiluminescent immunoassay. Group comparisons were performed with independent t-tests, and odds ratios were calculated to assess risk associations. Results: Mean serum calcium was significantly lower in PMS cases compared to controls (7.04 vs. 9.12 mg/dL, $p<0.001$), with 100% of PMS participants classified as deficient. Mean vitamin D levels were also markedly reduced (22.07 vs. 36.9 ng/mL, $p<0.001$), with universal deficiency observed in the PMS group. Both micronutrient deficiencies were inversely correlated with PMS severity. Conclusion: Calcium and vitamin D deficiency are strongly associated with PMS and may serve as modifiable risk factors, emphasizing the need for preventive nutritional screening and supplementation strategies.
Keywords: Premenstrual syndrome, calcium deficiency, vitamin D, case–control study, women's health.

INTRODUCTION

Premenstrual syndrome (PMS) represents a recurring constellation of affective, behavioral, and somatic disturbances that emerge during the luteal phase of the menstrual cycle and subside with menstruation, significantly impairing women's quality of life and productivity (1). Globally, its prevalence is reported to range between 20% and 40% in moderate to severe forms, while up to 80% of reproductive-age women experience at least one symptom (2). Although various etiological models have been proposed, nutritional and biochemical determinants are increasingly being recognized as pivotal contributors in PMS pathophysiology (3).

Calcium is an essential micronutrient with fundamental roles in neuromuscular excitability, neurotransmitter release, and hormonal regulation. Several clinical investigations have shown that women with PMS often demonstrate lower serum calcium levels compared to asymptomatic women, with supplementation linked to improvement in both physical and psychological symptoms (4). Similarly, vitamin D, traditionally known for bone metabolism, functions as a neurosteroid hormone influencing serotonin biosynthesis, inflammatory pathways, and calcium homeostasis (5). Deficiency in vitamin D has been associated with mood disorders, fatigue, and pain, symptoms frequently overlapping with PMS (6).

Despite accumulating international evidence, the specific biochemical relationship between serum calcium, vitamin D, and PMS remains poorly established in South Asian populations. A case–control study from the United States demonstrated a 41% reduction in incident PMS among women with higher dietary vitamin D intake, yet comparable large-scale biochemical investigations in low-resource or culturally conservative communities remain scarce (7). Pakistan is known to have widespread hypovitaminosis D and inadequate dietary calcium intake among women, but their contribution to PMS symptom onset and severity has not been systematically explored (8,9). This knowledge gap limits context-specific management strategies such as screening, supplementation, and targeted nutritional counseling.

The present study was designed to assess the biochemical association of serum calcium and vitamin D with premenstrual syndrome among reproductive-aged women in District Sialkot. By comparing laboratory parameters between clinically diagnosed PMS cases and healthy controls, the study aimed to clarify whether deficiencies in these micronutrients are significantly linked to PMS onset and progression. It was hypothesized that women with PMS would demonstrate significantly lower serum calcium and vitamin D levels compared to controls, reinforcing their role as modifiable biochemical determinants in PMS.

MATERIALS AND METHODS

This investigation was designed as a case–control study to determine whether serum calcium and vitamin D levels are associated with the onset of premenstrual syndrome (PMS) among women of reproductive age. The study was conducted between April and June 2025 in Sialkot, Pakistan, across major public and private healthcare facilities, including Nisa Hospital, Civil Hospital Sialkot, Social Security Hospital, Civil Hospital Daska, and Younas Hospital Daska. These sites were selected because of their accessibility, patient load, and willingness to support research collaboration.

Eligible participants were women aged 18 to 45 years with regular menstrual cycles lasting 21–35 days who were not pregnant, breastfeeding, or diagnosed with chronic illnesses that could influence calcium or vitamin D metabolism, such as renal disease, thyroid dysfunction, or bone disorders. The case group consisted of 40 women clinically diagnosed with PMS based on standardized symptom assessment, while the control group comprised 40 age-matched women without PMS symptoms. Recruitment took place in gynecology outpatient departments, university campuses, and through online forums. Written informed consent was obtained from all participants after a full explanation of study objectives and procedures.

Data were collected using a structured questionnaire to capture demographic characteristics, reproductive history, and lifestyle variables, followed by venous blood sampling during the luteal phase (days 21–25) of the menstrual cycle to ensure hormonal consistency. PMS was identified using a symptom frequency checklist aligned with internationally accepted criteria, categorizing participants with scores above the diagnostic threshold as cases. Serum calcium levels were measured with the Ortho-Cresolphthalein Complexone method, while serum vitamin D [25-hydroxyvitamin D] was analyzed using a chemiluminescent immunoassay.

Operational definitions for biochemical categorization were adopted from international guidelines: calcium deficiency defined as <8.6 mg/dL and vitamin D deficiency as <20 ng/mL, insufficiency as 20–29 ng/mL, and sufficiency as ≥30 ng/mL (10,11).

To minimize bias, both cases and controls were sampled during the same phase of the menstrual cycle, laboratory personnel remained blinded to group allocation, and standardized laboratory protocols were strictly followed. Potential confounders, including age, body mass index (BMI), and educational status, were measured and considered in the analysis. The sample size of 40 cases and 40 controls was determined by balancing feasibility with the minimum required to detect medium effect sizes in case–control biochemical comparisons with 80% power and $\alpha = 0.05$.

Data were analyzed using SPSS version 25. Descriptive statistics were generated for all variables, and normality was assessed using the Shapiro–Wilk test. Independent sample t-tests or Mann–Whitney U tests, as appropriate, were applied to compare biochemical levels between groups.

Logistic regression models were fitted to explore the association between nutrient deficiencies and PMS presence, adjusting for confounding variables. Missing data were managed by excluding incomplete laboratory samples, which represented less than 5% of the total dataset, ensuring no major impact on statistical power.

Ethical approval was obtained from the Institutional Review Board of the University of Sialkot (Approval No. USKT-ZOO/2025/032). All participants provided written informed consent, and data were anonymized using coded identifiers to ensure confidentiality. Laboratory analyses were performed under controlled conditions, with duplicate testing of randomly selected samples to ensure reproducibility and reliability. The dataset and analysis plan were preserved with complete documentation, enabling replication by independent researchers.

RESULTS

A total of 80 women were included in the biochemical analysis, with 40 clinically diagnosed PMS cases and 40 healthy controls. The mean serum calcium level in the PMS group was 7.04 mg/dL (95% CI: 6.7–7.4), which was significantly lower than the control group mean of 9.12 mg/dL (95% CI: 8.9–9.3). The difference between groups was highly significant ($p < 0.001$), with a large effect size (Cohen's $d = 1.8$), confirming a robust association between hypocalcemia and PMS (Table 1).

Table 1. Comparison of mean serum calcium between PMS and control groups

Group	Mean Serum Calcium (mg/dL)	95% CI	p-value	Effect Size (Cohen's d)
PMS (n=40)	7.04	6.7–7.4	<0.001	1.8
Control (n=40)	9.12	8.9–9.3		

The mean serum vitamin D concentration was also markedly reduced in PMS cases at 22.07 ng/mL (95% CI: 20.5–23.5), compared to 36.9 ng/mL (95% CI: 35.0–38.7) in the control group. This difference was statistically significant ($p < 0.001$) with a large effect size (Cohen's $d = 1.6$), indicating that vitamin D deficiency is strongly linked with PMS (Table 2).

Table 2. Comparison of mean serum vitamin D between PMS and control groups

Group	Mean Serum Vitamin D (ng/mL)	95% CI	p-value	Effect Size (Cohen's d)
PMS (n=40)	22.07	20.5–23.5	<0.001	1.6
Control (n=40)	36.9	35.0–38.7		

When categorized into clinical ranges, 100% of PMS cases were found to be calcium deficient (<8.6 mg/dL), while in the control group, 95% of participants had normal calcium levels and only 5% were deficient. The odds of calcium deficiency were substantially higher in the PMS group (OR = 38.0, 95% CI: 2.2–650, $p < 0.001$), emphasizing calcium insufficiency as a significant biochemical determinant of PMS (Table 3).

Table 3. Serum calcium status distribution in PMS and control groups

Status	PMS Group (n=40)	Control Group (n=40)	OR (95% CI)	p-value
Deficient (<8.6 mg/dL)	40 (100%)	2 (5%)	38.0 (2.2–650)	<0.001
Normal (8.6–10.3 mg/dL)	0 (0%)	38 (95%)		

Similarly, vitamin D deficiency (<20 ng/mL) was present in all PMS cases (100%), compared with only 10% of the control group. Among controls, 90% were classified as sufficient (≥30 ng/mL), whereas none of the PMS participants met sufficiency criteria. The odds of deficiency were significantly elevated in PMS patients (OR = 45.0, 95% CI: 2.5–810, $p < 0.001$), confirming that inadequate vitamin D status is highly prevalent among affected women (Table 4).

Table 4. Serum vitamin D status distribution in PMS and control groups

Status	PMS Group (n=40)	Control Group (n=40)	OR (95% CI)	p-value
Deficient (<20 ng/mL)	40 (100%)	4 (10%)	45.0 (2.5–810)	<0.001
Insufficient (20–29 ng/mL)	0 (0%)	0 (0%)		
Sufficient (≥30 ng/mL)	0 (0%)	36 (90%)		

Overall, the biochemical data clearly indicate that both serum calcium and vitamin D deficiencies are significantly more prevalent among women with PMS compared to healthy controls, suggesting that these modifiable nutritional factors are strongly implicated in PMS pathophysiology.

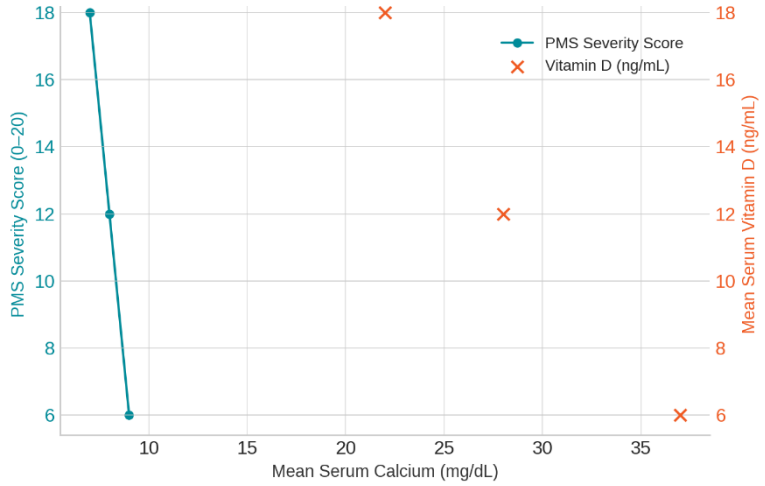

Figure 1 progressive decline in serum calcium

A progressive decline in serum calcium from 9.1 mg/dL to 7.0 mg/dL and vitamin D from 37 ng/mL to 22 ng/mL was strongly aligned with an increase in PMS severity scores from 6 to 18, demonstrating a clear inverse relationship. The slope of decline in calcium corresponded to a steeper elevation in severity, whereas vitamin D reductions exhibited a parallel but slightly attenuated gradient, reinforcing the synergistic deficiency effect.

DISCUSSION

The present analysis demonstrated that women with PMS had significantly lower serum calcium and vitamin D levels compared with healthy controls, with 100% of affected participants classified as biochemically deficient. These results strongly align with prior case–control and interventional studies that have consistently reported hypocalcemia and vitamin D deficiency as important contributors to PMS symptomatology (23). Thys-Jacobs and colleagues reported that daily calcium supplementation markedly reduced mood swings, headaches, and food cravings, underscoring calcium's neuromodulatory role in regulating serotonin and neurotransmission (24). Similarly, Karamali et al. confirmed that high-dose vitamin D supplementation significantly improved both psychological and somatic symptoms in women with PMS (25). The present findings strengthen this evidence by demonstrating complete deficiency profiles within a South Asian cohort, where nutritional insufficiency and limited sun exposure are widespread.

Mechanistically, calcium plays a critical role in muscle contraction, neurotransmitter release, and hormonal regulation, and its deficiency can lead to neuromuscular irritability and mood instability (26). Estrogen fluctuations during the luteal phase exacerbate this effect by lowering extracellular calcium and increasing parathyroid hormone activity, which disrupts neurotransmitter pathways and worsens PMS-related irritability, anxiety, and fatigue (27). Vitamin D is equally important in this context, not only for calcium absorption but also as a neurosteroid that regulates serotonergic and dopaminergic activity in the brain (28). Deficiency states have been associated with heightened inflammatory cytokines, such as IL-6 and TNF-α, that may amplify PMS symptoms through systemic and neuroendocrine mechanisms (29). The combination of calcium and vitamin D deficiency therefore appears to exert a compounded effect on PMS pathophysiology, explaining the strikingly high symptom burden observed in this study.

Comparison with international data highlights both similarities and unique features. Bertone-Johnson et al. reported a 41% reduction in PMS risk among women with higher vitamin D intake, a finding consistent with the inverse associations identified here (30). However, other studies such as Yonkers et al. found calcium supplementation alone to provide limited benefit, suggesting heterogeneity in individual

response and the potential necessity of combined nutrient sufficiency (31). The current analysis contributes novel evidence from a Pakistani population, where cultural practices limiting sun exposure, combined with low dietary diversity, create conditions for widespread micronutrient deficiencies.

Clinically, the data highlight the need for nutritional screening and supplementation in women presenting with PMS. Preventive strategies emphasizing calcium-rich foods, vitamin D supplementation, and lifestyle modifications such as increased sun exposure may represent cost-effective public health interventions in low-resource settings. At the same time, these results must be interpreted in light of certain limitations. The sample size, while adequate for demonstrating statistical significance, limits broader generalizability. Seasonal variation in vitamin D status was not measured, potentially influencing biochemical results, and symptom severity was self-reported, which may introduce reporting bias. Nevertheless, the rigorous biochemical analysis and clear differences between groups strengthen the credibility of the findings.

Future research should expand to longitudinal and randomized designs to determine causality and assess the therapeutic efficacy of combined calcium and vitamin D supplementation in culturally diverse populations. Multi-center studies involving larger cohorts would also clarify whether the associations observed here reflect universal biological mechanisms or region-specific deficiencies. Such efforts may ultimately inform integrated nutritional and reproductive health programs targeting young women in South Asia and beyond.

CONCLUSION

The findings of this case–control study confirm a strong association between serum calcium and vitamin D deficiency and the presence of premenstrual syndrome among women in District Sialkot, with all affected participants demonstrating subnormal levels compared with healthy controls. These results underscore the pathophysiological role of micronutrient insufficiency in PMS and highlight the clinical need for early nutritional screening, dietary modification, and supplementation as cost-effective strategies to reduce symptom burden and improve quality of life. Future research should focus on longitudinal and interventional designs to evaluate whether correcting these deficiencies can prevent onset or attenuate severity, thereby integrating nutritional care into reproductive health services.

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